University of Idaho
College of Agricultural and Life Sciences

Biological control of hoary cress (*Lepidium draba*): Pre-release studies and foreign exploration in Europe

Annual Report
Report period: 1 January – 31 December 2003

Mark Schwarzlaender, Jessica McKenney, Hariet Hinz*
and Michael Cripps

Department of Plant, Soil, and Entomological Sciences
University Of Idaho
Moscow, ID 83844-2339, USA

Phone 208-885-9319
Fax 208-885-7760
Email markschw@uidaho.edu

* CABI Bioscience Switzerland Center, Delémont, Switzerland

Funded by: **MoA AG2C5000513, Bureau of Indian Affairs, Rocky Mountain Regional Office, Billings, MT,; Idaho State Department of Agriculture 2003 Cost-Share Agreement, Big Horn Drainage Weed and pest Districts, Worland, WY; and Panhandle Lakes RC&D on behalf of the Hoary Cress Consortium.**

Table of Contents

Summary

1. We made good progress in 2003 studying hoary cress invasions in the western United States and developing biological control agents for this noxious weed. The Hoary Cress Consortium met its financial goals and the two graduate students (Michael Cripps and Jessica McKenney) continued their research overseas and in Idaho and adjacent states, respectively. Both have started to analyze their data and will graduate during 2004.

2. Foreign exploration results are briefly summarized in this report. However, our foreign exploration partner, CABI Bioscience will issue a detailed annual research report during February 2004. Since the beginning of the project in 2001, a total of 163 sites were sampled in 14 countries for potential biological control agents and ecological data, covering approximately 3,000 miles latitude and 1,300 miles longitude. Thirty of these field sites were studied quantitatively in detail using a standard protocol. Seven phytophagous insect species have been selected as potential biological control agents for hoary cress based on their restricted host range records, four weevils, one flea beetle, and two gall midges. During 2003, we concentrated our work on three of the weevil species and the flea beetle.

3. We finished the comparison of the growth vigor and herbivory pressure on hoary cress infestations between Europe and the western United States. Data will be evaluated over the next months. Results of this work indicate that hoary cress biomass is three times higher in North America compared to Europe but the biomass of individual shoots is not different. The higher hoary cress biomass in the U.S. results therefore from higher shoot densities. Insect herbivore numbers are similarly high for both continents. However, almost all insects in North America are polyphagous, they feed on many different plants. Most insects found at European hoary cress infestations are specialists, they feed only on hoary cress and potentially on a few closely related crucifer species.

4. Dr. John Gaskin at the USDA ARS Northern Plain Agricultural Research Laboratory, Sidney, MT, continued to review the taxonomic situation of the genus *Lepidium*. He conducted genetic studies including all field sites studied by the two graduate students in the U.S. and Europe. There are several different genotypes occurring in the U.S. indicating that hoary cress has been introduced several times from different places.

5. The test plant species list for the assessment of the host-specificity of biological control agents for hoary cress currently developed by Linda Wilson (University of Idaho) and Jeff Littlefield (Montana State University, Bozeman, MT) with contributions by Hariet Hinz and John Gaskin is nearly completed. New molecular studies on Brassicaceae will soon be published and we want to include that information in the test plant list prior to publication.

6. Hoary cress and perennial pepperweed are now in the same genus *Lepidium*. We have decided to broaden the scope of the hoary cress consortium and include perennial pepperweed (*L. latifolium*) and dyers woad (*Isatis tinctoria*) in the foreign exploration effort. This will however require additional funding efforts by all groups interested in such a biocontrol program.

1 Introduction

The three known hoary cress or whitetop species are no longer in the genus *Cardaria.* New taxonomic studies conducted in the U.S. and Germany using molecular techniques revealed that the genus *Cardaria* is no longer valid. The genus was reunited with the genus *Lepidium.* In addition, there are only two and not three hoary cress species. *Cardaria draba* (L.) Desv. (heart-podded hoary cress) is now *Lepidium draba* L. The name *Cardaria pubescens* (C.A. Meyer) (globe-podded hoary cress) Jarmolenko has changed to *Lepidium appelianum* Al-Shehbaz because a *L. pubescens* already exists in North America. *Cardaria chalepensis* (lens-podded hoary cress) is no longer an independent species. It is now considered a subspecies of heart-podded hoary cress. For more detailed information on the name changes of hoary cress, please refer to the CABI Bioscience 2002 annual report.

Hoary cress species currently infest large areas of pasture, rangeland and riparian habitat in Washington, Oregon, Idaho, Montana, Wyoming, Utah, California, and Alberta. They can also cause serious problems in grains such as alfalfa and wheat and some orchard crops. Hoary cress serves as an early season host plant for the cabbage seed-pod weevil (*Ceutorhynchus obstrictus*), a major pest of CANOLA and oilseed rape in Alberta and plant bugs in the genus *Lygus,* the most important pest insect in alfalfa seed production. Hoary cresses are declared noxious weeds in 14 western states and three Canadian provinces.

Hoary cress species are deep-rooted, perennial mustards with stout stems that grow up to 60 cm tall (Fig. 1). The root system consists of persistent vertical and lateral roots from which new rosettes and flowering shoots arise, thus allowing the plants to develop into thick stands. The root system makes cultural control efforts impractical. Established clones survive thatching treatments and repeated cutting or cultivation. Grazing is not a promising control technique because sheep do not graze established clones. Cattle avoid hoary cress and when forced to graze it, produce tainted milk. Successful control has been achieved with metasulfuron and 2,4-D, but chemical control is considered uneconomic because of the large areas infested and large size of individual infestations.

Hoary cress species are native to western and central Asia, southeastern Europe and the Mediterranean region. They were probably introduced to the New World in the late 19th century in contaminated alfalfa seed, and plants were first noted around seaports along both East and West coasts.

Mustard weeds are generally considered difficult candidates for biological control because they are closely related to many important crop plants. In addition, there is a large number of closely related North American Brassicaceae, some of which are considered rare, threatened or endangered. However, the severity of the problems caused by hoary cress species, and the current difficulties to effectively manage the noxious weed species has led to a biological control program. Wyoming Weed and Pest Districts, the Idaho State Department of Agriculture (ISDA), and the University of Idaho started to investigate the feasibility of biocontrol of hoary cress species in 2001. In 2002, Idaho Fish and Game, the USDI BLM, Vale, OR, and USDI BIA, Billings, MT joined the consortium. In 2003 more partners, the Oregon Department of Agriculture, Salem, OR, the Blaine County Weed Management Area, and Ada County (ID) were welcomed in the consortium.

Fig. 1 Hoary cress (*Lepidium draba*) infestation on the Crimea Peninsula, Ukraine.

2 CABI Bioscience work in 2003

The results of the foreign exploration efforts conducted by the CABI Bioscience Switzerland Centre will be presented in a separate detailed annual report issued in February 2004. This report will be sent to all consortium partners and upon request (markschw@uidaho.edu) to anyone interested. The following is a summary of the 2003 foreign exploration accomplishments.

Field surveys 2003 During five field trips, conducted between 29 April and 29 May 2003, 24 sites were sampled quantitatively in Switzerland, Germany, Austria, Hungary, Romania, Bulgaria, and the Ukraine (see Fig. 7). Eight of these had already been sampled in 2002. Plant and insect data were collected using the same protocol as in 2002. In addition, 13 samples of *L. draba* collected in Bulgaria and the Ukraine were sent to Dr. John Gaskin (USDA ARS NPARL, Sidney, MT) for genetic analysis. Between 21 June and 23 July, all sites were revisited to collect data on seed and biomass production. However, samples could only be taken from 20 of the 24 sites, since the two sites in Switzerland had been mown, and two sites in Romania had been grazed. As last year, many plants had already senesced and shed a large proportion of their leaves. To be able to correct for this underestimation of biomass, we therefore took the biomass of remaining leaves and shoots separate this year.

To date, seven phytophagous insect species have been selected as potential biological control agents for hoary cress based on records of their restricted host range, i.e. four weevils, one flea beetle and two gall midges. During 2003, we concentrated our work on three weevil species and the flea beetle.

Ceutorhynchus cardariae **(Coleoptera, Curculionidae)** Adults of this gall forming weevil (Fig. 2) were obtained from our collaborator Dr. Alecu Diaconu from Romania in 2002, and overwintered on potted *L. draba* plants in the Centre's garden. On 1 March 2003, first eggs were found. Galls are initiated through female oviposition and are mostly formed at petioles and stems. Heavily galled shoots are stunted and may even die. To collect data on the host-specificity and biology of *C. cardariae,* we established sequential no-choice oviposition and development tests. We were able to infest 141 plants, 66 *L. draba* and 20 test species with 1-5 replicates each, including nine native North American plants. Galls and/or eggs were found on five test species, adults emerged from three, unfortunately including the indigenous *Stanleya pinnata* and *Thlaspi montanum* var. *montanum*. However, none of the other seven indigenous species exposed were attacked, including two native *Lepidium* species and *Stanleya viridiflora*. It will be important in 2004 to conduct additional no-choice tests with other indigenous *Thlaspi* species (e.g. *T. idahoense*) and single or multiple-choice development tests with *S. pinnata* and *T. montanum*. Several hundred adults emerged from infested control plants during June and July. Newly emerged adults feed and then aestivate over summer. About one third of females have a short oviposition period in late summer/early autumn. In conclusion, *C. cardariae* is easy to rear, shows good potential for fast population increase, has the potential to damage the plant and so far exhibits a relatively restricted host range. It is therefore a priority agent we will concentrate on in 2004.

Fig. 2 *Ceutorhynchus cardariae*: Adult on hoary cress shoot.

Ceutorhynchus merkli **(Coleoptera, Curculionidae)** Work on this stem-mining weevil continued in 2003 (Fig. 3). Adults were collected in early spring by our collaborator Dr. Stefan Töpfer in Hungary and shipped to Delémont to conduct host-specificity tests.

During single-choice oviposition tests conducted in 2002 and 2003, 20 of 30 plant species offered were accepted. Therefore female oviposition behavior seems to be not very specific and these tests were therefore discontinued. During sequential no-choice oviposition and development tests using potted plants with 26 plant species, ten were mined, including the indigenous *Lepidium virginicum, Stanleya pinnata* and *Streptanthus farnsworthianus*. However, in *L. virginicum* and *S. farnsworthianus*, only 0.7% and 2.1% of the total shoot lengths were mined, respectively. Apart from the three target weeds, i.e. *Lepidium draba* spp. *draba, L. draba* spp. *chalapense* and *L. appelianum,* adults emerged thus far only from *Brassica nigra* and *Thlaspi arvense*. Adult emergence from control plants was poor in 2003, likely due to the extremely hot and dry weather in summer. In addition, mines on test plants could also have been caused by other weevil species naturally occurring in the Centre garden. Therefore, tests will be repeated in 2004. We will also try to improve adult emergence rates using different techniques.

Fig. 3 *Ceutorhynchus merkli:* Adult on leaf.

***Ceutorhynchus turbatus* (Coleoptera, Curculionidae)** *Ceutorhynchus turbatus* is a seed-feeding weevil with one generation per year (Fig. 4). It is widespread and abundant and was thus far found at all field sites. It is described as monophagous on *L. draba*, however, due to difficulties to obtain consistent female oviposition in captivity, we have not been able to confirm its restricted host range. On 1 May 2003, nearly 1,000 *C. turbatus* adults were collected within 30 minutes at a field site in southern Switzerland and subsequently used in no-choice oviposition and development tests with nine test plants. Shoots with flowers and developing seeds were covered with gauze bags and 14-15 weevils each released. After 2-3 weeks, a subsample of fruits was dissected and signs of feeding, oviposition or larval mining recorded. Similar to 2002, few eggs were laid in test and control plants. Feeding holes of adults were found on four of the nine plant species offered, i.e., *L. campestre, Sinapis alba, Isatis tinctoria,* and *Cardamine pratensis*. In 2004, we will try again to increase female oviposition rates.

Fig. 4 *Ceutorhynchus turbatus:* Adult of the seed feeding weevil on hoary cress buds.

***Psylliodes wrasei* (Coleoptera, Chrysomelidae)** A *Psylloides* flea beetle that had already been observed in 2001 by our Romanian collaborator Dr. Alecu Diaconu has recently been identified as *Psylloides wrasei* (Fig. 5). Little is known about the biology and host range of this species. However, preliminary tests conducted by a group of scientists in Russia indicated a narrow host range of this potential biological control agent for hoary cress. During a field trip to Romania at the end of March 2003, we found one field site at which up to 80% of *L. draba* plants were heavily attacked by first instars of *P. wrasei*. According to Dr. Diaconu, the larvae can kill developing shoots of *L. draba.* Adults reared through at the Centre emerged at the end of May/beginning of June. In addition, we received a shipment with over 600 *P. wrasei* adults from Dr. Diaconu. Beetles were kept in cylinders or on potted plants to investigate their

biology and conduct preliminary host-specificity tests. On 14 June, no-choice adult feeding tests were established with the commercially grown *Brassica oleracea capitata,* the closely related *L. squamatum, L. perfoliatum* and *Isatis tinctoria,* the North American *Lepidium medium*, and the control, *L. draba*. Hardly any feeding occurred on any of the test plants offered, while all controls were attacked or showed heavy signs of feeding. According to our experience, feeding of adult flea beetles is usually not very specific. We therefore assess these preliminary results as indication for a very selective host range of this new potential agent. Feeding ceased by the end of June and recommenced at the beginning of August. On 26 August, first eggs were found, and until 28 October, a total of 1,691 eggs were laid. 1,096 eggs were transferred into petri dishes. No larvae have hatched from eggs yet. We assume that eggs go through a diapause as is known for other flea beetle species. The fact, that we found first instars in field collected plants at the end of March is another indication for this diapause. In addition, 540 eggs were transferred onto potted plants of *L. draba* (n=16), which are being overwintered in the Centre garden. Plants will be re-examined in spring 2004. Provided, larvae will hatch from eggs, we will start no-choice larval transfer tests in 2004 and continue studies on the biology and life cycle of this very promising potential agent species.

Fig. 5 *Psylloides wrasei:* Adult of the root-mining flea beetle on hoary cress leaf.

Other potential biocontrol agent species Depending on available funding, a field trip to southern Kazakhstan will be organized during 2004 to obtain specimens of the two gall midge species *Contarinia cardariae* and *Dasyneura cardariae*. Both gall midges have recently been described for the first time. They are reported to feed exclusively on hoary cress.

3 Research at University of Idaho

Studies on the biology of hoary cress invasions were continued at the University of Idaho during 2003. A second field season of data was collected for the comparison of hoary cresses growth between Europe and the United States. With these studies we try to assess plant growth and vigor and the amount in composition of insect herbivory between weed populations in their native and introduced ranges. New field sites were surveyed during 2003 and a number of sites surveyed during 2002 were revisited. Consecutive sampling in two years will allow comparison of plant vigor and herbivory between the years and provide a measure for fluctuation of hoary cress invasion magnitude and insect feeding. We also tried to expand the geographical range of field sites that were surveyed. In the U.S. 19, sites were visited during 2003 in Idaho, Oregon, Washington and Wyoming (Fig. 6). In Europe, 24 sites were visited in the Ukraine, Bulgaria, Switzerland, Austria, Germany, Romania, and Hungary (Fig.7).

Fig. 6 Hoary cress field sites (black stars) surveyed in the U.S. during the 2003 field season.

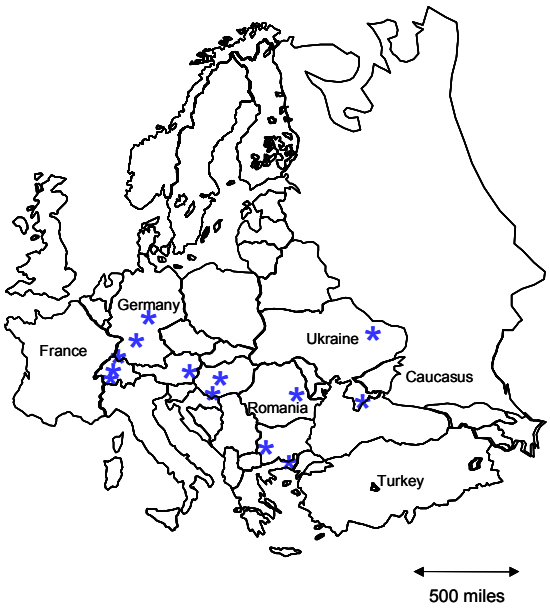

Fig. 7 Hoary cress field sites surveyed in Europe during the 2002- 03 field seasons.

Preliminary results of plant growth parameters confirm the trends found in 2002. The percentage cover for *L. draba* was significantly lower at sites in Europe compared to the U.S. (Fig. 8). Similarly, the percentage cover for plant competitors was twice as high at European sites. In contrast to results from 2002, there were no significant differences in the percentage cover for bare ground between field sites in Europe and the U.S. (Fig.8).

Hoary cress shoot densities continued to be smaller at European field sites in 2003 (Fig. 9). The number of shoots at field sites in the U.S. was twice as high (Fig. 9). At all field sites surveyed in 2003, we also collected data on shoot biomass. However, respective samples are still being analyzed. Thus, data is not yet available. There is however, indication that the differences in hoary cress growth between field sites in the U.S. and Europe can be explained through the shoot density. In 2002, there were no significant differences in the average shoot biomass between European and U.S. populations.

Similar to 2002, shoot attack was determined through dissection of individually sampled shoots. For each site (Europe and U.S.) approximately 180 shoots per site were dissected in the laboratory under a stereomicroscope. Insect presence or attack was noted. Similar to the 2002 results attack rates were higher for European hoary cress infestations (Fig. 10).

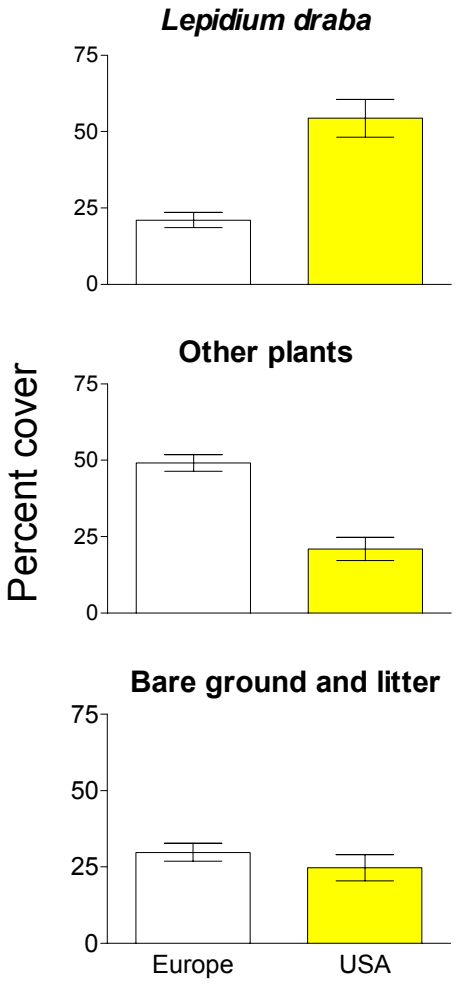

Fig. 8 Percent cover of *Lepidium draba* ($F_{1, 40}$ = 24.99***), other plant species ($F_{1, 40}$ = 36.58 ***), and bare ground ($F_{1, 40}$ = 0.92 *n.s.*) at field sites in Europe (white bars) and the United States (yellow bars) in 2003.

In 2003, we also recorded soil parameters at all field sites that were visited. We are particularly interested in the soil nitrogen content. We are currently analyzing available nitrogen pool, bulk density, and particulate organic matter for field sites in Europe and North America at the University of Idaho in collaboration with Dr. John Lloyd. The analysis of soil samples will allow us to explain the role of soil nutrient condition in hoary cress invasions in North America.

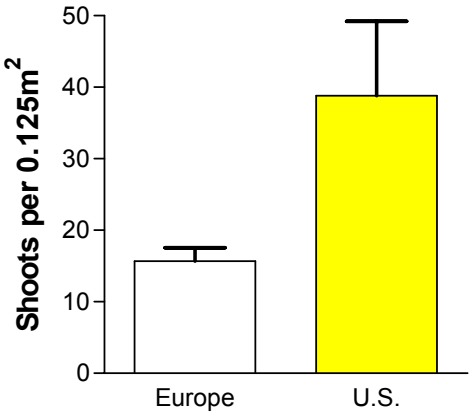

Fig. 9 Mean density of hoary cress shoots on a 25cm by 12.5 cm area at field sites in Europe (white bars) and the United States (yellow bars) in 2003. Differences are highly significant ($F_{1, 40} = 19.05$***).

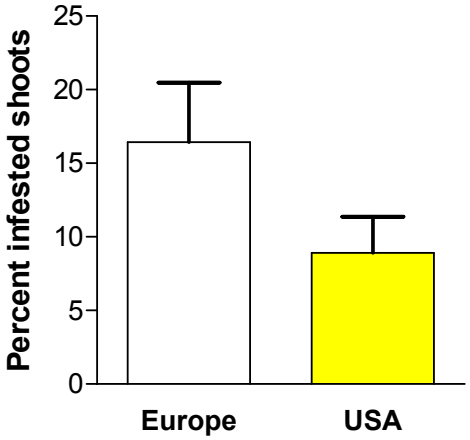

Fig. 10 Percentage attacked hoary cress shoots at field sites in Europe (white bars) and the United States (yellow bars) in 2003.

We are currently conducting a controlled greenhouse experiment at the University of Idaho to study the growth vigor of European and North American hoary cress biotypes under standardized conditions. We are particularly interested to see whether U.S. biotypes of hoary cress will produce more biomass when they are grown under the exactly the same conditions as European biotypes. If this would be the case, it would be an indication that hoary cress has changed genetically since its introduction into North America.

Preliminary results for hoary cress growth parameters and herbivory in 2003 are consistent with those from last year. Data analyses still continues for plant and herbivory data from the 2003 and for herbivory data from the 2002 field season. We have also only started to compare data between years. The comparison of hoary cress infestations in Europe and North America will provide us with a good descriptive account on the biology of this noxious weed. We will know the extent to which the plant species is more aggressive in the U.S. which is a first step to start more analytical studies that explain the precise reasons for the competitiveness of hoary cress in the western United States.

We accomplished two important goals during the 2003 field season. We managed to increase the geographical range that was surveyed on both continents and we increased the number of field sites visited. We visited sites in Wyoming, central Oregon and Washington in the U.S. and moved east in Europe as far as the Ukraine. The analyses of tall collected data will provide us detailed quantitative assessment of the current and potential future problems caused by hoary cress invasions. This work will allow us to identify key factors responsible for hoary cress invasions. Based on the findings of this study, we hope to receive competitive USDA funding that would allow us to test those identified key factors using manipulative experiments.

4 Conclusions

This was the third year of the hoary cress consortium and although a lot of data has not been completely analyzed, we think that we have accomplished a lot during these three summers. In our efforts to develop a comprehensive biological control program for hoary cress in North America, we have accomplished all major goals outlined in the strategic plan with limited funding sources. The foreign exploration process is at an unusually advanced stage with host-specificity tests started for three agents. Our pre-release studies have provided us with a very good understanding of the abundance of potential biocontrol agents in

the field and some of the important life stages of hoary cress for which we have to find biological control agents.

There are six potential biological control agents identified, four of which are already reared and studied for their biology at the CABI Bioscience Switzerland Centre. In addition, we have found one North American stem-mining weevil with unknown host range and unknown biology that we want to study because it heavily attacks hoary cress at one field site in Oregon. Host-specificity tests will continue to be conducted in 2003. The test plant species list is completed but kept unpublished until a new publication on Brassicaceae taxonomy can be reviewed. The test plant list is comprehensive but it has the advantage that it can be used for hoary cress, perennial pepperweed and dyers woad.

Genetic studies showed that there are several different hoary cress genotypes introduced in North America, some originating in Europe and others that only occur in western Asia (John Gaskin, USDA ARS NPARL, Sidney, MT, personal communication). Studies comparing the growth vigor of these different genotypes under standardized conditions are underway at the University of Idaho. These studies will allow us to identify the most and least 'aggressive' hoary cress genotypes in the western United States.

Appendix Sponsors and funding provided and used in 2003

Project Sponsors	CABI Bioscience Funding		University of Idaho Funding	
	Received	Purpose	Received	Purpose
Idaho State Department of Agriculture	$25,000	Foreign exploration in central & eastern Europe Preliminary studies on biology of potential agents, Switzerland	$29,000	Graduate Student, M.S., Comparison study U.S.
Wyoming Weed and Pest Districts	$41,500			
Idaho Fish and Game	*pending*			
USDI–BLM Vale, OR	*pending*			
USDI–BIA			$10,000	Graduate Student, M.S., Comparison study Switzerland, Europe
Univ. of Idaho Stipend			$14,500	
Univ. of Idaho USDA-NRI Grant			$38,560	Graduate student, M.S., Pre-release studies in U.S.
Blaine County CWMA	$12,250			
Oregon Department of Agriculture, ODA	*pending*			
Ada County, ID	$1,000			Graduate Student, M.S., Comparison study U.S.
Total	**$79,750**		**$92,060**	